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Telomere Length in Circulating Leukocytes Is Associated with Lung Function and Disease

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Take home message

Telomere length, a marker of biological age, is decreased in asthma and COPD patients and positively associated with spirometric indices.

Abstract

Several clinical studies suggest the involvement of premature aging processes in COPD. Using an epidemiological approach we studied whether accelerated aging indicated by telomere length, a marker of biological age, is associated with COPD and asthma, and whether intrinsic age-related processes contribute to the inter-individual variability of lung function.

Our meta-analysis of 14 studies included 934 COPD cases with 15,846 controls defined according to GLI criteria (or 1,189 COPD cases according to GOLD), 2,834 asthma cases with 28,195 controls, and spirometric parameters (FEV₁, FVC and FEV₁/FVC) of 12,595 individuals. Associations with telomere length were tested by linear regression, adjusting for age, sex, and smoking status.

We observed negative associations between telomere length and asthma ($\beta=-0.0452$, $p=0.024$) as well as COPD ($\beta=-0.0982$, $p=0.001$), with associations being stronger and more significant when using GLI in comparison to GOLD. In both diseases, effects were stronger in women compared to men. The investigation of spirometric indices showed positive associations between telomere length and FEV₁ ($p=1.07 \times 10^{-7}$), FVC ($p=2.07 \times 10^{-5}$), and their ratio FEV₁/FVC ($p=5.27 \times 10^{-3}$). The effect was somewhat weaker in apparently healthy subjects compared to COPD or asthma patients.

Our results provide indirect evidence for the hypothesis that cellular senescence may contribute to the pathogenesis of COPD and asthma and that lung function may reflect biological aging primarily due to intrinsic processes which are likely to be aggravated in lung diseases.

Key words: biological age, premature aging, COPD, asthma, spirometry

Introduction

An increasing life expectancy is associated with an increased susceptibility for prevailing chronic diseases, such as cardiovascular and neurological diseases, type 2 diabetes and cancer, suggesting that common, age-related processes are involved in these diseases [1-4]. The lung is known to undergo age-related processes associated with a continuous decline in lung function [5, 6]. Chronic obstructive pulmonary disease (COPD), lung cancer and idiopathic pulmonary fibrosis are lung diseases with an increase in incidence by age. In COPD, chronic inflammation and oxidative stress are among the generally accepted pathophysiological mechanisms but, as discussed for other degenerative diseases, accelerated aging resulting in premature cell senescence may contribute to the pathobiology [7-9]. Although clinical and epidemiological studies provide some evidence for this hypothesis the data base is still limited [8] so that our study aims to provide further clues on this.

Telomeres made up of the simple repetitive sequence TTAGGG in humans [10] are specialized DNA structures which in concert with telomere specific proteins protect the ends of chromosomes and maintain the integrity of the genome [3]. They progressively shorten with each cell division [11] and after reaching a critical length, either cellular senescence or apoptosis is induced [3]. Telomeres are therefore considered as a marker of biological age. Their length is affected by various factors, such as chronic inflammation or oxidative stress [3], but has also been shown to have a heritable component [12]. Recent data suggest that shorter telomere length is associated with an increased risk of total and cancer mortality in COPD patients [13]. Shortened telomeres were found in pulmonary vascular endothelial cells, alveolar epithelial cells and circulating leukocytes of COPD patients [14-18] supporting the notion of accelerated aging in COPD. Rode et al. [18] reported multivariable-adjusted odds ratios of 1.15 (95% CI 1.06 to 1.25) for shortest *versus* longest telomere quartiles for COPD. Smoking, a major cause of COPD, is associated with different signs of premature aging which

is particularly obvious in the skin [8] suggesting that environmental exposure may play an important role in triggering processes related to biological aging.

Since the lung is continuously exposed to environmental hazards, lung function per se may be a surrogate marker for biological age in light of the large inter-individual variability observed [19]. Only a few case-control studies [14, 20] have investigated the relationship between telomere length and spirometric indices. One large population based study was recently published by Rode et al. [18] reporting a modest correlation between telomere length and lung function in terms of the spirometric indices forced expiratory volume in one second (FEV₁), forced vital capacity (FVC) and their ratio FEV₁/FVC.

In a meta-analysis of 14 European studies embedded in the ENGAGE consortium, we studied the relationship between telomere length, lung function and lung disease to address the question whether accelerated aging indicated by telomere length may be involved in the pathogenesis of COPD and asthma, and whether intrinsic age-related processes may contribute to the inter-individual variability of lung function. For this purpose, we explored the association between circulating leukocyte telomere length and COPD or asthma. Although these are different obstructive disease entities, they share chronic inflammation as a hallmark of disease which may result in accelerated aging and telomere shortening. We further investigated the association between telomere length and lung function using the spirometric indices FEV₁, FVC and FEV₁/FVC.

Methods

Study populations

This meta-analysis comprises data from 14 European studies, with details provided in the **Online Data Supplement**. In the analysis of telomere length and COPD, we combined data

of nine studies (EX-ATHLETES, FITSA, KORA Age, KORA F3, KORA F4, NAG-FIN, NFBC1966, TwinGene, and TwinsUK), totaling 934 cases and 15,846 controls (**Table 1**).

COPD was defined as $z(\text{FEV}_1/\text{FVC}) < -1.6445$ (5% lower limit of normal (LLN) according to the Global Lung Function Initiative (GLI) [19]) if spirometry was available (see below), or based on physician diagnosis otherwise. In a sensitivity analysis we replaced the GLI criteria by the GOLD criteria ($\text{FEV}_1/\text{FVC} < 0.70$) [21] to define COPD. The analysis of telomere length in asthma comprised 13 studies (ERF, EX-ATHLETES, FITSA, GRAPHIC, KORA Age, KORA F3, KORA F4, NAG-FIN, NFBC1966, NTR, QIMR, TwinGene, and TwinsUK), with 2,834 cases and 28,195 controls (**Table 2**). Asthma was defined by physician diagnosis or self-reports in questionnaires or by asthma-specific medication intake. Spirometric measurements (FEV_1 , FVC and FEV_1/FVC) were available for 12,595 individuals from seven studies (FITSA, KORA Age, KORA F3, KORA F4, NFBC1966, TwinFat, and TwinsUK). Spirometry had been performed in line with international standards [22, 23]. Relative telomere length was determined by qPCR [24] in all studies. Measurement details by study are provided in the **Online Data Supplement**. The local ethic committees had approved all studies and all participants had given their written informed consent.

Statistical analysis

Associations of telomere length with COPD, asthma, FEV_1 , FVC and FEV_1/FVC were tested by linear regression models with telomere length as outcome in all models. To account for heterogeneity between studies, telomere length was standardized within each study prior to the analysis, using a z-transformation after initially adjusting for age and sex, i.e. scaling the residuals of the regression of telomere length on age and sex to zero mean and unit variance. COPD and asthma cases, respectively, were coded "1", whereas controls were coded "0". Also the spirometric indices were transformed to z-scores taking into account age, height, and gender of each individual. Therefore Global Lung Function Initiative (GLI) reference

equations for spirometry [19] were applied to calculate z-scores for FEV₁, FVC, and FEV₁/FVC from skewness (L), the coefficient of variation (S) and the predicted value (M) according to the LMS-equations by using the provided software (<http://www.lungfunction.org/tools>).

Within the linear regression model we adjusted for ever and current smoking, as well as for study specific covariates as case/control status or family structure if appropriate. As both, telomere length and lung function measurements were already adjusted for age and sex, the association model itself did not contain these covariates. All analyses were primarily conducted in all available study samples and additionally conducted stratified by sex. The z-transformation of telomere length was performed in males and females separately for the sex-stratified analysis. For the analyses of FEV₁, FVC, and FEV₁/FVC, we additionally stratified into the subgroups “apparently lung healthy”, “COPD cases” and “asthmatics”.

The group of apparently lung healthy subjects was determined by exclusion of all subjects with a history of asthma, COPD, or other reported respiratory diseases, and all subjects with a reported acute respiratory tract infections within the last three weeks. The group was further divided into “current/former” and “never” smokers. In the group of healthy smokers we further investigated subjects showing the lower and upper 25%-quantiles of lung function (FEV₁, FVC or FEV₁/FVC respectively). The single study results were combined in a meta-analysis with inverse variance weighting, performed in the statistical software R (<http://www.r-project.org>) using the meta-package with fixed and random effects. As a measure for heterogeneity, I² values were calculated [25] and Cochran’s heterogeneity test was performed. As a sensitivity analysis in COPD, we excluded first all subjects over 70 years and then all subjects over 60 years from the analysis.

Results

COPD

By combining nine studies including 934 COPD cases and 15,846 controls according to GLI (**Table 1**), we observed a significant negative association between COPD and telomere length ($\beta=-0.0982$, $p=0.001$ with fixed effects; $\beta=-0.0953$, $p=0.012$ with random effects, $I^2=27.7\%$, $p_{\text{het}}=0.198$). With COPD defined by GOLD criteria there were more COPD cases ($n=1,189$) with an increase in older and a reduction in younger individuals. On the other hand, the association with telomere length using GOLD criteria was more heterogeneous across studies and less significant ($\beta=-0.0676$, $p=0.018$ with fixed effects, $\beta=-0.0629$, $p=0.117$ with random effects; $I^2=42.3\%$, $p_{\text{het}}=0.085$). **Figure 1** shows study specific and combined effect estimates of a fixed and random effects model with COPD defined by GLI. The association was strong in the female subsample ($n=10,435$; $\beta=-0.1306$, $p=3.6 \times 10^{-4}$ with fixed effects; $\beta=-0.1312$, $p=6.7 \times 10^{-4}$ with random effects; $I^2=5.8\%$, $p_{\text{het}}=0.385$) whereas in the male subsample no significant relationship was detectable ($n=6,345$; $\beta=-0.0242$, $p=0.663$ with fixed effects; $\beta=-0.0329$, $p=0.622$ with random effects; $I^2=23.5\%$, $p_{\text{het}}=0.250$). **Figure E1** shows the meta-analysis stratified for sex. The two studies showing positive effect estimates, EX-ATHLETES and NAG-FIN, are selective in their study design: NAG-FIN comprises families of ever heavy smokers, and the EX-ATHLETES are former male top athletes with matched controls and a mean age of 72.7 years. Since COPD shows an age-related increase in incidence, we conducted sensitivity analyses to investigate whether the observed association is affected by age. Exclusion of subjects >70 years or >60 years showed no significant differences for the effect estimates in subgroups of both sexes (**Figure E2**), confirming that the association is not driven by older age groups.

Asthma

By combining 13 studies with 2,834 asthma cases and 28,195 controls (**Table 2, Figure 2**), we observed a negative association between telomere length and asthma. This was significant in a fixed effects model ($\beta=-0.0452$, $p=0.024$) and suggestive in a random effects model ($\beta=-$

0.0446, $p=0.093$; $I^2=30.4\%$, $p_{\text{het}}=0.140$). The effect was driven by the female subsample ($n=18,724$, $\beta=-0.0764$, $p=0.002$ with fixed effects; $\beta=-0.0809$, $p=0.005$ with random effects; $I^2=15.8\%$, $p_{\text{het}}=0.289$) and not seen in the male subsample ($n=12,305$; $\beta=0.0182$, $p=0.594$ with fixed effects; $\beta=0.0139$, $p=0.771$ with random effects; $I^2=40.3\%$, $p_{\text{het}}=0.080$; **Figure E3**).

Lung function

By combining data of seven studies, totaling 12,595 individuals (**Table 3, Figure 3**), we observed highly significant positive associations between telomere length and spirometric indices measured as FEV₁ ($\beta=0.0455$, $p=1.07 \times 10^{-7}$ with fixed and random effects; $I^2=0\%$), FVC ($\beta=0.0401$, $p=2.07 \times 10^{-5}$ with fixed and random effects; $I^2=0\%$), and their ratio FEV₁/FVC ($\beta=0.0238$, $p=5.27 \times 10^{-3}$ with fixed and random effects; $I^2=0\%$). The effect for FEV₁ was higher in women compared to men ($\beta=0.0548$ versus $\beta=0.286$ with random effects). The meta-analysis stratified by sex is shown in **Figure E4**.

Figure 4 shows the association results stratified by lung health status. Compared to “healthy”, we found somewhat stronger effects in the disease subgroups of COPD and asthma cases. A comparison of the effect estimates stratified by health status as well as by sex is presented in **Figure E5**. The stronger effects found in COPD were predominantly seen in men and less obvious in women. The opposite was observed for asthma, where the effects were strong in women. With regard to smoking, within the healthy subgroups, the observed effects were stronger in “healthy never smokers” compared to “healthy ever smokers” for FEV₁ and FVC (see **Figure E6**). To further explore the contribution of smoking we stratified the group of healthy smokers (current or ex) by lung function status (lower 25% or upper 25% within this subgroup). The results suggest that there was no difference between those two groups for FEV₁. In FVC, an effect was only seen in individuals with poor lung function (lower 25%). Although differences between subgroups were noticeable, they did not differ significantly.

Discussion

We conducted a large study investigating the relationship between telomere length and lung function and disease, assessed in multiple European cohorts unselected for lung disease. We found highly significant positive associations between telomere length from peripheral leukocytes and spirometric indices measured as FEV₁, FVC, and FEV₁/FVC in a meta-analysis of seven studies with 12,595 individuals. When stratifying by lung health status, larger effects were seen in the subgroups of COPD patients or asthmatics. This suggests that lung function decline in part reflects biological aging due to intrinsic processes which are likely to be aggravated in asthma or COPD due to mechanisms promoting cell senescence.

Tobacco smoke is a well-known environmental factor which promotes aging processes and cellular senescence [8]. The recent large, population-based study by Rode et al. (2013) [18] revealed that having ever smoked and the cumulative pack-years smoked were associated with telomere length in 46,396 adults. This is in line with previous population-based findings [26]. However, in clinical studies these effects were not seen [14, 17, 20]. When stratifying by smoking status, we observed no evident associations between telomere length and lung function indices in ever smokers. Also stratifying by lung function within the group of healthy smokers (current and ex) did not clarify the issue, although for FVC an effect could be detected in smokers of the lower quartile. The underlying reason for detecting no effect may be found in the complex interactions between smoking and genetic and/or life style factors, such as antioxidant diet or physical activity, affecting biological age and its markers [1, 3, 27-32], but also in the heterogeneity of smoking exposures, its amount and duration.

Up to now, a limited number of studies have addressed the association between lung function and telomere length. Mui et al. (2009) [20] observed a positive association in a sample of COPD patients, and Tsuji et al. (2006) [16] reported that airflow limitation and alveolar cell senescence were positively correlated with each other. Other studies failed to find significant associations between spirometric parameters and telomere length [14, 17, 33], although one study found that parameters of gas exchange (arterial oxygen and carbon dioxide pressures) were associated with telomere length in COPD patients [14]. The large population-based study by Rode et al. (2013) found weak but statistically significant positive correlations between telomere length and spirometric indices, which is confirmed by our results. Furthermore we observed the effects to be somewhat stronger in COPD or asthma patients compared to healthy individuals, suggesting that intrinsic age-related processes may be aggravated by chronic inflammatory processes occurring either locally in the lung or systemically.

In both, COPD and asthma patients, we observed shorter telomeres compared to healthy controls. In COPD, the association was stronger when defining cases by the age dependent lower limit of normal for FEV₁/FVC according to GLI [19] compared to the fixed cut off given by GOLD [21]. This suggests that the GLI approach better suits the transition from health to disease. For COPD and asthma, effect estimates appeared to be larger in females than in males although sex differences were not statistically significant. With respect to COPD, this might be consistent with the observation that females appear to be more susceptible to COPD than males [34] but the causality certainly needs to be proved. Whereas our findings for asthma are novel, the results for COPD are in line with previous findings in case-control [14-17] and population-based studies [18]. This suggests that, as already shown for other chronic diseases [1, 3, 35], accelerated aging may contribute to the pathogenesis of asthma and COPD.

Our epidemiological finding is supported by results from clinical studies showing shorter telomeres in alveolar epithelial and endothelial cells from patients with emphysema [16]. Amsellem et al. reported a higher percentage of pulmonary vascular endothelial cells stained for senescence markers in COPD patients than in lung healthy controls [15]. This is in line with senescent-type growth characteristics of lung fibroblasts [36]. Furthermore, cellular senescence of pulmonary smooth muscle cells appears to be involved in the pathogenesis of pulmonary hypertension in COPD [37]. Several mechanisms have been proposed for abnormal aging in COPD including chronic inflammation, oxidative stress, mitochondrial dysfunction, altered energy metabolism, abnormal regulation of aging processes, cellular and immunosenescence, and telomere dysfunction [3, 7-9, 15]. Analogous mechanisms in asthma have not been explored in detail but due to the inflammatory nature of both diseases some of the above mentioned factors are likely to also play a role in asthma. Possible consequences for both diseases are very difficult to predict but one might speculate that the aging processes may be reflected in the shorter life expectancy and an increased risk for cognitive impairment and dementia as reported for asthma and COPD [38-40].

The observed associations between telomere length and both diseases were not as strong as one might have initially expected, considering the large sample size included. In case of COPD, in the population-based approach of Rode et al. [18] also a much weaker association was observed than previous case-control studies had suggested [14]. In our case this may be related to the fact that mostly mild to moderate disease stages were included in the study samples. E.g. for COPD in the KORA cohorts GOLD stages I and II covered 94.3% of COPD cases. Rode et al. also observed weaker effects for stages I (OR=1.09, 95% CI 0.97 to 1.23) and II (OR=1.17, 1.04 to 1.31) compared to more advanced GOLD stages [18] after multiple

adjustment. In the case of asthma, the weakness of association could be due to the different methods of ascertaining asthma cases or phenotypic heterogeneity.

Moreover, as in many other studies, telomere length has been measured in circulating leucocytes and not in the cells of the primarily affected organ, i.e. the lung. Studies addressing the comparability of telomere length between different tissues found strong correlations in healthy individuals, but somewhat blunted ones in disease [41-44]. We cannot rule out that this may be a factor which affects the association of telomere length with lung diseases. Leukocyte telomere length is a complex trait that is shaped by many factors including genetic, epigenetic, lifestyle and environmental determinants, and their complex interaction is hardly understood. In the present analysis we therefore adjusted only for well accepted determinants. Certainly, the population-based epidemiological nature of the studies included comes along with distinct limitations. Population-based samples typically only scarcely include severe cases of lung diseases but predominantly early disease stages, in which age-related mechanisms may not be as significant as in more advanced stages that are primarily included in clinical cohorts [14-17]. Furthermore, our study design did not allow taking into account the known heterogeneity of COPD and asthma phenotypes. These entities may have different impacts on telomere length or may trigger premature aging processes via telomere-independent mechanisms as reported for fibroblasts of patients with lung emphysema [45]. Unlike in clinical studies, diagnosis of COPD and asthma had to rely mainly on self-reported physician diagnosis which certainly adds some potential for misclassification error. On the other hand, we employed the recent GLI reference values [19] for COPD diagnosis wherever possible. Also confounding effects, such as smoking or medication, could not be accounted for in the same way as a matched case-control study design can.

As this analysis combines a number of studies which are heterogeneous in their design (see **Online Data Supplement**), we report results of random effects models in addition to the results based on fixed effects in order to account for this heterogeneity. Naturally, confidence intervals are larger with random effects. While both models reach statistical significance in COPD, statistical significance was only reached with the fixed effects model for asthma. The observed effect point estimates, however, were comparable between the fixed and the random effects model, supporting the biological significance of our findings. In COPD, our sensitivity analysis did not show an age-dependency (**Figure E2**). Two studies showing positive effect estimates for COPD were selective in their study design; in case of the EX-ATHLETES the positive effect of life-time physical exercise on biological age may have played a role [3, 27, 46, 47].

Conclusions

We investigated the relationship of telomere length, as a marker of biological age, with lung function and respiratory disease. We found highly significant positive associations between telomere length and lung function. Furthermore, shorter telomeres were seen in patients with COPD and asthma cases compared to healthy controls. Our results provide indirect evidence for the hypothesis that premature aging is involved in the pathophysiology of COPD and asthma and that lung function partially reflects biological aging due to intrinsic processes. These processes are likely to be aggravated by lung diseases promoting cellular senescence, although the average effect seems to be not particularly strong.

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A detailed list of acknowledgments is provided in the **Online Data Supplement**.

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Figure legends

Figure 1: Associations between telomere length and COPD

Forest Plot comparing effects between studies, and showing combined effects in a fixed effects model as well as in a random effects model. Sex-stratified results are based on random effects models. 95% confidence intervals are given for all estimates. COPD was defined by GLI criteria.

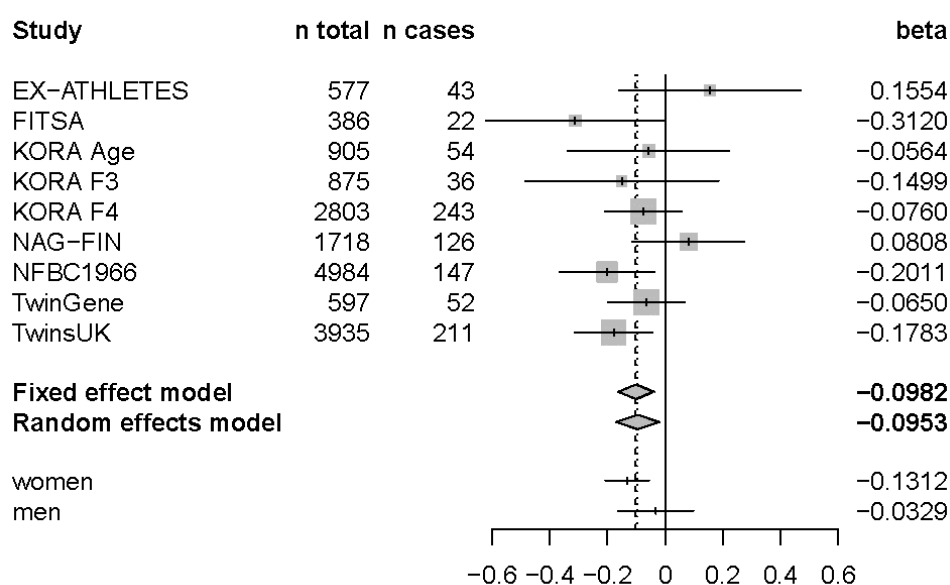


Figure 2: Association between telomere length and asthma

Forest Plot comparing effects between studies, and showing combined effects in a fixed effects model as well as in a random effects model. Sex-stratified results are based on random effects models. 95% confidence intervals are given for all estimates.

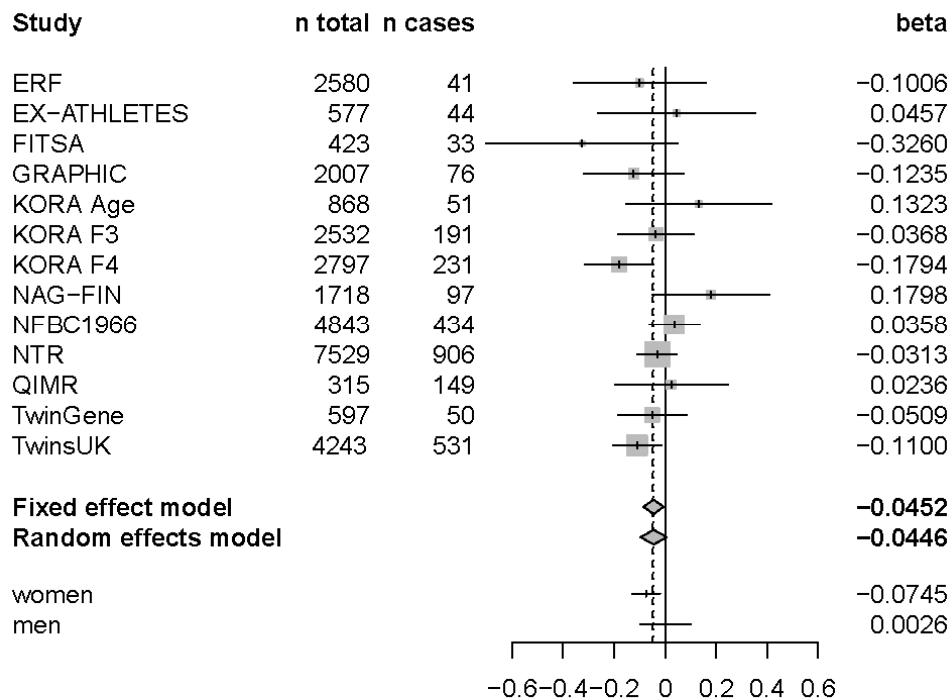


Figure 3: Telomere length is associated with FEV₁, FVC and (FEV₁/FVC)

Forest Plot comparing effects between studies, and showing combined effects in a fixed effects model as well as in a random effects model. Sex-stratified results are based on random effects models. 95% confidence intervals are given for all estimates.

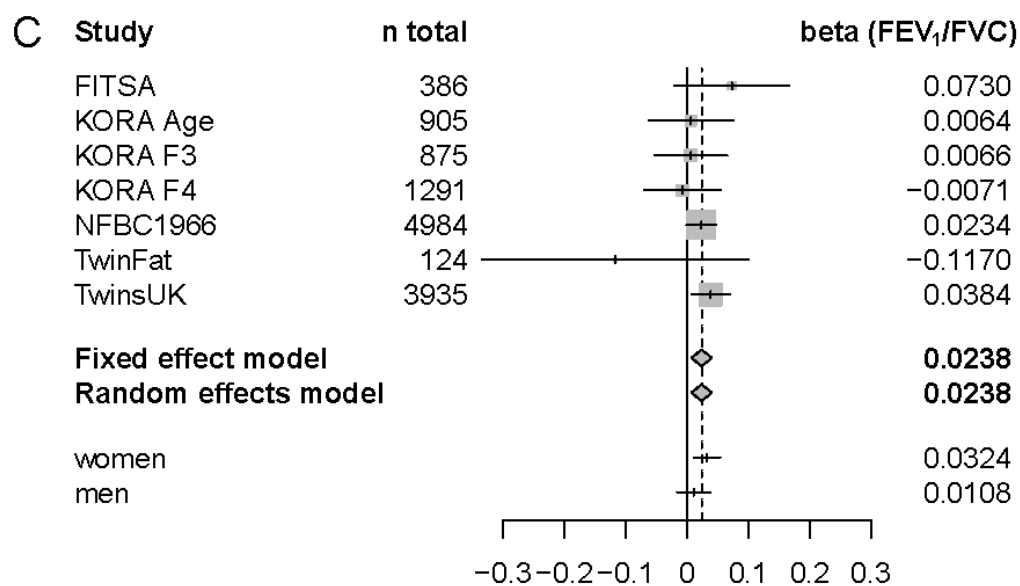
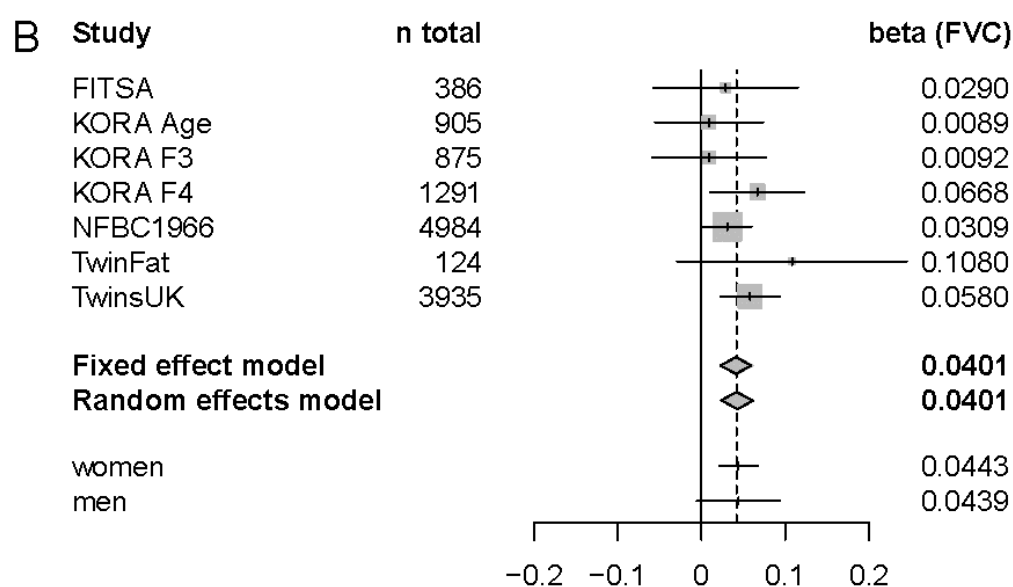
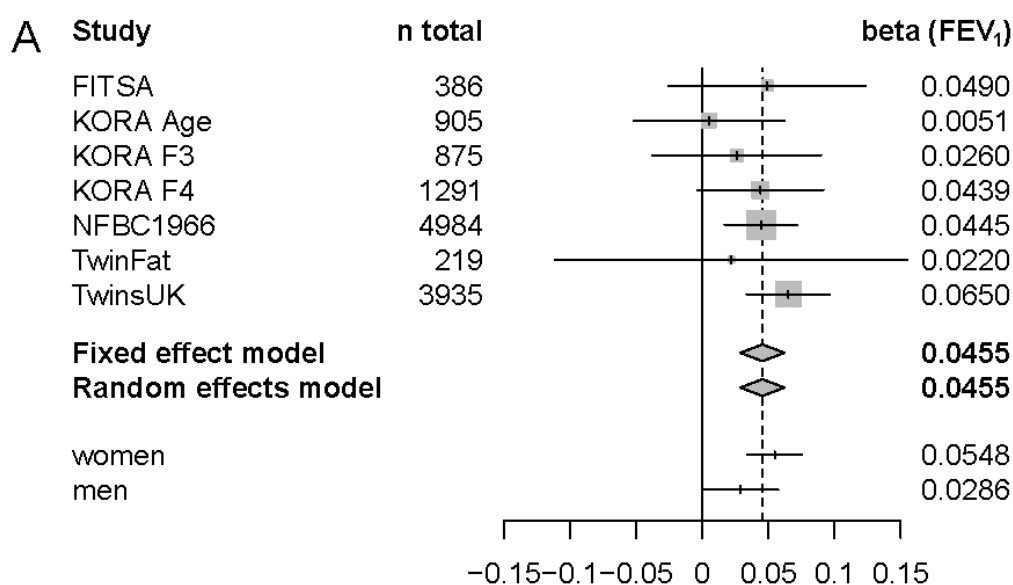
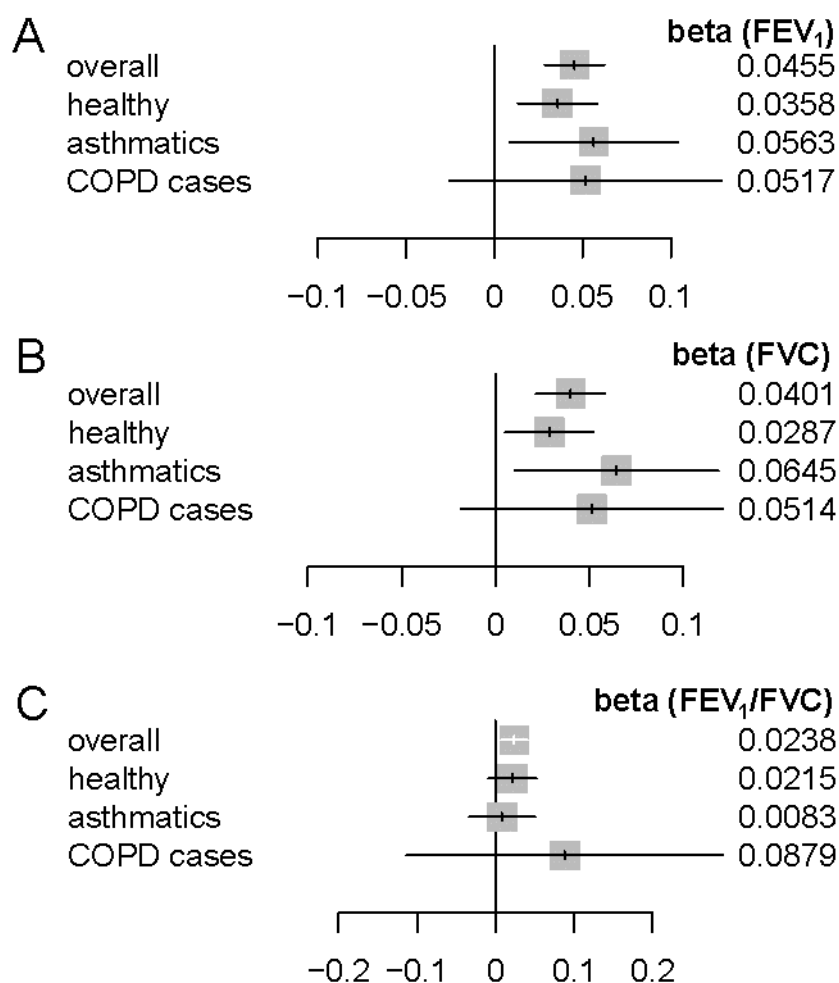


Figure 4: Associations between telomere length and FEV₁, FVC and (FEV₁/FVC), stratified by health status.

Forest Plot showing effects in the overall sample as well as in “apparently lung healthy” individuals only, for asthmatics, and patients with COPD to test the impact of lung disease on the effect. Effects are based on random effects models. 95% confidence intervals are given for all estimates.



Tables

Table 1: Study characteristics of studies in COPD analysis defined by GLI criteria

Study	total sample size	number of COPD cases	number of women	Smoking status never/former/current	Age [years] mean (sd) overall	Age [years] mean (sd) in cases	Age [years] mean (sd) in controls	Age diff
EX-ATHLETES	577	43 (7.5%)	0 (0%)	361/177/39	72.68 (5.92)	74.63 (6.97)	72.52 (5.81)	2.11
FITSA	386	22 (5.7%)	386 (100%)	333/31/21	68.45 (3.38)	69.27 (3.60)	68.40 (3.36)	0.87
KORA Age	905	54 (6.0%)	451 (49.8%)	510/351/44	75.67 (6.34)	75.02 (6.28)	75.71 (6.35)	-0.69
KORA F3	875	36 (4.1%)	458 (52.3%)	377/260/238	44.45 (8.93)	44.11 (9.57)	44.46 (8.91)	-0.35
KORA F4	2,803	243 (8.7%)	1,480 (52.8%)	1,230/1,048/525	54.86 (12.84)	59.35 (12.73)	54.44 (12.77)	4.91
NAG-FIN	1,718	126 (7.3%)	782 (45.5%)	204/804/710	55.89 (7.63)	58.40 (9.40)	55.69 (7.44)	2.71
NFBC1966	4,984	147 (2.9%)	2,594 (52.0%)	2,479/592/1,913	31 (0.2)	31 (0.2)	31 (0.2)	0
TwinGene	597	52 (8.7%)	597 (100%)	358/239*	71.72 (5.89)	72.50 (5.31)	71.65 (5.95)	0.85
TwinsUK	3,935	211 (5.4%)	3,687 (93. 7%)	2,760/743/432	51.17 (13.12)	53.34 (13.06)	51.02 (13.14)	2.32
total	16,780	934 (5.6%)	10,435 (62.2%)					1.71[#]

*for TwinGene the numbers correspond to current smoking no/yes. [#]mean age difference between cases and controls, weighted by total sample size.

Table 2: Study characteristics of studies in asthma analysis

Study	total sample size	number of asthma cases	number of women	Smoking status never/former/cur rent	Age [years] mean (sd) overall	Age [years] mean (sd) in cases	Age [years] mean (sd) in controls	Age diff
ERF	2,580	41 (1.6%)	1,468 (56.9%)	981/691/908	49.80 (15.67)	51.14 (13.35)	49.78 (15.71)	1.36
EX-ATHLETES	577	44 (7.6%)	0 (0%)	361/177/39	72.68 (5.92)	73.23 (5.98)	72.63 (5.92)	0.60
FITSA	423	33 (7.8%)	423 (100%)	369/32/21	68.61 (3.41)	68.27 (3.48)	68.64 (3.41)	-0.37
GRAPHIC	2,007	76 (3.8%)	994 (49.5%)	1,109/491/407	39.31 (14.50)	37.09 (14.20)	39.38 (14.51)	-2.29
KORA Age	868	51 (5.9%)	435 (50.1%)	494/332/42	75.65 (6.34)	76.02 (6.10)	75.63 (6.36)	0.39
KORA F3	2,532	191 (7.5%)	1,314 (51.9%)	1,133/863/536	53.62 (10.8)	55.77 (9.85)	53.44 (10.86)	2.33
KORA F4	2,797	231(8.3%)	1,477 (52.8%)	1,228/1,045/524	54.90 (12.85)	54.15 (12.75)	54.97 (12.85)	-0.82
NAG-FIN	1,718	97 (5.6%)	782 (45.5%)	204/804/710	55.89 (7.63)	59.72 (9.07)	55.67 (7.48)	4.05
NFBC1966	4,843	434 (9.0%)	2,516 (52.0%)	2,463/590/1,790	31 (0.2)	31 (0.2)	31 (0.2)	0
NTR	7,529	906 (12.0%)	4,688 (62.3%)	3,734/2,213/1,582	42.50 (15.29)	41.51 (14.80)	42.64 (15.35)	-1.13
QIMR	315	149 (47.3%)	161 (51.1%)	NA	20.94 (5.48)	20.60 (5.40)	21.25 (5.56)	-0.65
TwinGene	597	50 (8.4%)	597 (100%)	358/239*	71.72 (5.89)	71.69 (7.56)	71.73 (5.73)	-0.04
TwinsUK	4,243	531(12.5%)	3,869 (91.2%)	3,003/784/456	51.07 (13.39)	49.09 (14.20)	51.37 (13.25)	-2.28
total	31,029	2,834 (9.1%)	18,724 (60.3%)					-0.27[#]

*for TwinGene the numbers correspond to current smoking no/yes. [#] mean age difference between cases and controls, weighted by total sample size.

Table 3: Study characteristics of studies in lung function indices analysis

Study	FITSA	KORA Age	KORA F3	KORA F4	NFBC1966	TwinFat	TwinsUK
Sample size	386	905	875	1,291	4,984	219	3,935
number of women	386 (100%)	451 (49.8%)	458 (52.3%)	691 (53.5%)	2,594 (52.0%)	101 (46.1%)	3,687 (93.7%)
Age [years] mean (sd)	68.45 (3.38)	75.67 (6.34)	44.45 (8.93)	51.62 (5.73)	31 (0.2)	27.60 (1.98)	51.17 (13.12)
FEV ₁ [l] mean (sd)	2.20 (0.49)	2.49 (0.72)	3.56 (0.82)	3.34 (0.82)	3.95 (0.79)	4.03 (0.84)	2.74 (0.66)
FVC [l] mean (sd)	2.82 (0.59)	3.36 (0.93)	4.3 (1.02)	4.30 (1.01)	4.72 (0.99)	4.74 (1.12)*	3.41 (0.73)
FEV ₁ /FVC mean [%] (sd)	78.19 (8.29)	74.32 (8.42)	83.09 (7.08)	77.65 (6.16)	84.11 (6.43)	84.98 (6.07)*	80.42 (8.47)
zFEV ₁ mean (sd)	0.19 (1.34)	0.35 (1.17)	0.17 (1.06)	0.21 (1.16)	0.21 (1.01)	-0.03 (1.09)	-0.09 (1.13)
zFVC mean (sd)	0.13 (1.19)	0.47 (1.01)	-0.06 (0.98)	0.33 (0.97)	0.11 (0.95)	-0.29 (1.04)*	-0.09 (0.98)
zFEV ₁ /FVC mean (sd)	-0.54 (1.13)	-0.26 (0.97)	0.42 (1.13)	-0.28 (0.89)	0.15 (1.13)	0.20 (0.96)*	-0.02 (1.12)
Smoking status never/former/current	333/31/21	510/351/44	377/260/238	496/496/299	2,479/592/1,913	110/54/55	2,760/743/432

*In TwinFat the analysis of FVC and FEV₁/FVC is based on a subsample of n=124 subjects.